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High dilutions of two drugs induce changes in crystal water structure of lactose as revealed by thermogravimetry and differential scanning calorimetry

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Ultra-high dilutions (UHD) of drugs used in homeopathy are preserved in sugars. How do solid sugars assimilate characteristics of UHDs? This study attempts to answer this question. The three UHDs of Acid fluoric, Acid nitric, and ethanol were mixed with lactose at 1 µL/g and analyzed by thermogravimetry (TG) and differential scanning calorimetry (DSC) to see any change in their crystal water. TG results show a mass loss of 4.9% at 146.8°C for Acid fluoric 30 cH, of 7.1% at 146.6°C for Acid nitric 30 cH, and5.1% at 146.5°C for ethanol. DSC results show that the change in enthalpy for Acid fluoric 30 cH is 157.3 J/g at 153.8°C, that for Acid nit 30 cH is 122.8 J/g at 148.3°C, and that for ethanol is 154.9 J/g at 156.3°C. Bound water in lactose crystals and corresponding enthalpies vary markedly in the three drugs tested. This indicates that the number of hydrogen bonds and their bond strength vary inbound water of medicated lactose crystals.

Keywords: Bound water, Enthalpy, Lactose, Homeopathic drugs, Hydrogen bond

UHDs of drugs used in homeopathy are preserved in sugars, particularly lactose and sucrose, which are thought to carry the information of original drugs. Hydrogen-bonded crystal water in sugar plays an important role in molecular recognition process[1,2]. Can crystal water in lactose medicated with different UHDs show any variation?. Molecular recognition, both inter and intra-molecular, plays a very important role in almost all biological processes[3]. This study is designed to address this question. UHDs used in homeopathy are called potencies. In a series of experiments, we demonstrated that homeopathic potencies differ from each other with respect to free water molecules and hydrogen bond strength of OH groups[4-7]. Besides free water homeopathic potencies also induce changes inbound water molecules associated with the water of crystallization or crystal water in lactose[8]. Lactose ($C_{12}H_{22}O_{11}$), a disaccharide of galactose and D-glucose, has been traditionally used as a standard medium for dispensing homeopathic potencies. Lactose tested here is composed of both α and β-forms having melting points at 223 and 252°C (Wikipedia retrieved on 05.08.2016)[7]. In the present study we observed the effect of the 30th potency of Acid fluoric and Acid nitric, and their medium ethanol on the crystal water of lactose by TG as well as DSC. Ethanol itself is also used as a homeopathic drug[9]. All three drugs tested have the same amount of water 99.91% and ethanol 0.09%.

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Materials and Methods

Acid nitric 30 cH (Lot: 11171N43624D), Acid fluoric 30 cH (Lot: 1081IN43412D), both products of Dr. Reckeweg & co., Germany were purchased in sealed vials from the local market at Kolkata. Absolute ethanol of Merc. KGaA, Germany (Index no.-603-002-00-5) was purchased in a sealed bottle from the local market at Kolkata. The two drugs in 90% ethanol *v/v* were mixed with DD water in the proportion of 1:1000 *v/v*. Absolute ethanol was first mixed with DD water to make it 90%, which was further diluted with DD water 1:1000. Thus ethanol and water content of all the three test drugs were 0.09% and 99.91%, respectively. Lactose was purchased from SRL, Mumbai. Each diluted test drug was mixed with lactose in the proportion of 1 µL drug/1 g lactose.

Thermogravimetry (TG)

Each medicated lactose sample was put into an alumina crucible. The sample and reference crucibles were placed inside the thermogravimetry analyzer

(STA 449F3 Jupiter of Netzsch, Germany). The instrument measures free and bound water in the test samples in terms of mass loss as a function of temperature. Free water evolves first at a temperature below 100°C, but bound water requires a higher temperatures for its removal[10]. In the present study, we wanted to observe only the bound water. After loading the sample into the analyzer, the analyzer was allowed to stabilize (for 30 min) at room temperature (30°C) underflow of dry nitrogen. Then the sample was heated at the rate of 10°C min^{-1} up to 200°C and any variation of sample mass was recorded with increasing temperature. During the period of stabilization under dry nitrogen gas flow, free water is usually evolved away. UHP-N_2 (99.999%) was used as the protective gas in the instrument and also as pure gas for the prevention of oxidation of the sample. The variation of the derivative of thermogravimetry data with temperature (differential thermogravimetry, DTG) indicates the rate of change of mass with temperature. The mass loss in this study is due to dehydration and not due to the decomposition of the material tested within 200°C because lactose melts at much higher temperature[8].

Differential Scanning Calorimetry (DSC)

Each test sample was put into an aluminium sample pan of 5 mm diameter which was sealed by a sealing machine. The weight of the test samples in each pan varied from12-15 mg. Differential Scanning Calorimetry (DSC) of the samples was measured, one at a time, by an instrument NETZSCCH DSC 200 F3 Maia, Germany at a scanning rate of 10°C min^{-1} in the temperature range 26°-200°C. A vacant pan was measured first as a reference. Thermograms and changes in enthalpy of the three samples were measured following the standard methods[11,12].

Results

TG

The TG curve shows a mass loss of 4.9% (solid line) at a temperature of 146.8°C in the case of Acid fluoric 30. The DTG curve (dotted line) shows the rate of change of temperature corresponding to mass loss (Fig. 1). In the case of Acid nitric 30, the mass loss was 7.1% as revealed by the TG curve. The DTG curve shows the corresponding temperature at 146.6°C (Fig. 2). In the case of ethanol the TG curve shows a mass loss of 5.1%. The DTG curve shows the rate of change of temperature corresponding to the mass loss at 146.5°C (Fig. 3).

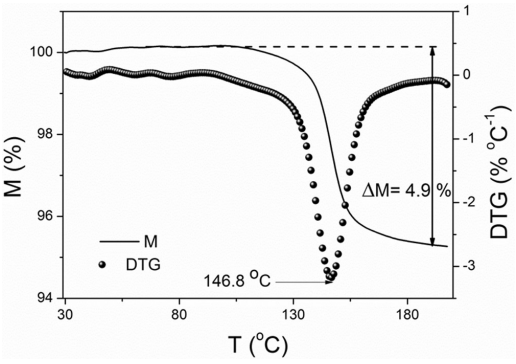

Fig. 1 — Thermogravimetric (TG) analysis curve (solid line) showing a loss in weight of lactose mixed with Acid fluoric 30 cH in 0.09% EtOH at 1 µL/g lactose. Derivative of thermogravimetry (DTG) curve (dotted line) showing the rate of change of weight with temperature

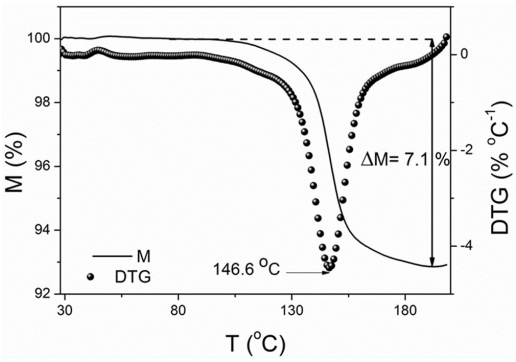

Fig. 2 — TG curve (solid line) showing a loss in weight of lactose mixed with Acid nitric 30 cH (in 0.09% EtOH) at 1 µL/g lactose. DTG curve (dotted line) showing the rate of change of weight with temperature

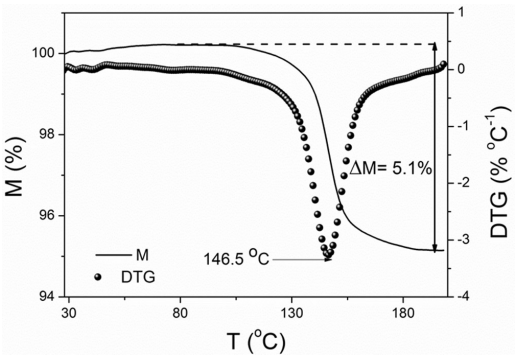

Fig. 3 — TG curve (solid line) showing a loss in weight of lactose mixed with aqueous ethanol (0.09%) at 1 µL/g lactose. DTG curve (dotted line) showing the rate of change of weight with temperature

DSC

The DSC curve shows the release of bound crystal water from lactose samples at 153.8°C for Acid fluoric 30, 148.3°C for Acid nitric 30, and 156.3°C for ethanol (Fig. 4). The area of the peak indicates

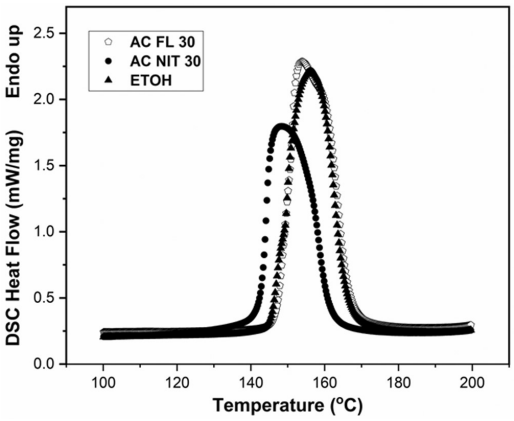

Fig. 4 — Differential scanning calorimetry (DSC) curves showing the endothermic change in enthalpy of lactose samples mixed with Acid fluoric 30 cH (157.3 J/g), Acid nitric 30 cH (122.8 J/g) and ethanol (154.9 J/g), at 1 µL/g lactose. All the three test drugs were in 0.09% EtOH. Enthalpies are shown in parentheses

a change in enthalpy. Here, the enthalpies were 157.3 J/g for Acid fluoric 30, 122.8 J/g for Acid nitric 30, and 154.9 J/g for ethanol.

Discussion

This experimental study shows that water molecules associated with drug-soaked lactose crystals vary in amount with respect to the nature of the drugs. DSC is used for the analysis of crystals of mixtures of drugs. The results indicate changes in the physical properties of the test samples[13]. The potencies of the two drugs were the same, 30 cH, and ethanol content in all the three samples was also the same, 0.09%. In our earlier studies, we reported that homeopathic potencies differ from each other with respect to free water molecules and hydrogen bond strength of the OH groups[4-7]. This study shows that homeopathic drugs also differ from each other with respect to bound water molecules, and this difference is confirmed by TG and DSC. DSC further shows that different quantities of thermal energy were needed to release bound water from medicated lactose crystals (Fig. 4). This means that the strength of the binding of crystal water molecules also varied with different drugs. So, crystal water in medicated sugars assumes different structure according to the nature of the drugs. Beckett *et al.*(2006) observed changes in enthalpies at 150°C in crystalline sucrose obtained from different sources. Mineral salts were reported to be responsible for this change[14]. It is reported that H^+ and OH^- play a role in strengthening the hydrogen bonding structure of water-ethanol[15]. The solubility of α-lactose in a mixture of water -ethanol increases with temperature and with water concentration[16]. Solubility releases crystal water. In our work crystal water varied in spite of constancy in water content. Obviously, it was the effect of the potentization of drugs by the special process of successive dilution followed by mechanical agitation or succussion.

We have mentioned that hydrogen bonding plays an important role in the differentiation of homeopathic potencies[4-7]. In fact, hydrogen bonding controls crystallization, structure, and packing of polymers[17]. Bound water molecules could directly control the specificity and affinity of binding between a protein and three types of sugars. This study was based on highly refined atomic structures of the complexes formed of the protein and three sugars[1]. Water molecules after gaining access into a carbohydrate, such as lactose, can replace a weak intra-molecular interaction by two stronger hydrogen bonds. This leads to marked changes in conformational preferences of the carbohydrate for binding[2]. Hydrogen bonded water molecules contribute significantly to carbohydrate molecular recognition processes[2]. Homeopathic potencies are structured water molecules designed by their original drug molecules during their preparation by successive dilution with succussion (potentization) when specific hydrogen bonding may occur. The information imbibed by a potency from the original drug in the liquid state is transferred to sugar molecules during medication in the form of changes in crystal water structures. A patient's proteins at the site of application of a drug on oral mucosa may recognize the structure in the medicated sugar resulting in further action of the drug on the patient. Crystal water structure in medicated sugars is stable and helps in prolonged storage without deterioration of the medicinal property. Ethanol-water dimer is a good model system for hydrogen bonding because it shows both strong O─H•••O hydrogen bond and also a weak C─H•••O hydrogen bond. Weak hydrogen bonds maintain a directional preference. These weak interactions are ubiquitous influencing molecular crystallization, macromolecular structure, and drug-receptor recognition. Each homeopathic potency is characterized by free OH groups, hydrogen bond strength, and a number of hydrogen bonds. All these 3 factors influence crystal water in lactose[18].

Conclusion

UHDs of Acid nitric and Acid fluoric induce changes in bound water of lactose crystals. The

changes involve amount of crystal water and the strength with which the water molecules are tightly bound. The amount of energy needed to break free the bound water in two UHDs is independent of the amount of bound water.

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